Christmann, et al.

Application No.: 09/654,293

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### IN THE CLAIMS:

Please amend Claim 1 as follows:

- 1. (Amended) A method of producing a reconstructed avian zygote or oocyte comprising the steps of:
- (i) providing a recipient cell selected from the group consisting of avian oocytes arrested at metaphase II and pronuclear zygotes;
- (ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;
  - (iii) enucleating the recipient cell; and
- (iv) introducing a donor nucleus from the same species as the recipient cell into the recipient cell to produce a the reconstructed avian zygote or oocyte.
- 2. The method of claim 1, in which the nuclear material of the recipient cell is visualized with near-infrared light using two photon laser scanning microscopy.
- 3. The method of claim 1, wherein the light has a wavelength from about 700 nm to about 1000 nm.
- 4. The method of claim 1, wherein the recipient cell is enucleated through the use of laser-mediated ablation.

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Please amend claim 5 as follows:

- 5. (Amended) The method of claim 1, wherein the visualization and enucleation are conducted using two photon laser scanning microscopy.
- 6. The method of claim 1, wherein the donor nucleus is genetically modified.

Please cancel Claims 7-10.

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### Please amend Claim 11 as follows:

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11. (Amended) The Athod of claim 1 wherein the avian is selected from the group consisting of chickens, ducks, turkeys, quails, ostriches and pheasants.

Please cancel Claims 12 and 13.

# Please amend Claim 14 as follows:

- 14. (Amended) A method of producing a cloned avian comprising the steps of:
- (i) providing a recipient cell selected from the group consisting of avian oocytes arrested at metaphase II and pronuclear zygotes;
- (ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;
  - (iii) enucleating the \*ecipient cell using light in the near infrared region;
- (iv) introducing a donor nucleus from the same species as the recipient cell into the recipient cell to produce a reconstructed avian zygote or oocyte;
  - (v) activating the reconstructed zygote or fertilizing the reconstructed oocyte;
- (vi) transferring the reconstructed zygote or fertilized oocyte into an oviduct of a recipient female of the same species as the zygote or oocyte; and
  - (vii) allowing the reconstructed zygote or oocyte to develop to term.
- 15. The method of claim 14, wherein the light has a wavelength from about 700 nm to about 1000 nm.
- 16. The method of claim 14, wherein the recipient cell nucleus is visualized using two photon laser scanning microscopy.
- 17. The method of claim 14, wherein the recipient cell is enucleated using two photon laser scanning microscopy.

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The method of claim \,\dagger{4}\,\text{ wherein the visualization and enucleation are conducted} 18. using two photon laser scanning microscopy.

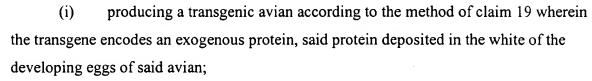
# Please amend Claim 19 as follows:

- 19. (Amended) A method of producing a transgenic avian comprising the steps of:
- providing an avian recipient cell selected from the group consisting of avian oocytes arrested at metaphase II and pronuclear zygotes;
- visualizing the nuclear material of the recipient cell using light in the nearinfrared region;
  - enucleating the recipient cell; (iii)
- introducing a transgenic avian donor nucleus from the same species as the (iv) recipient cell into the recipient call to produce a reconstructed avian zygote or oocyte;
  - activating the reconstructed zygote or fertilizing the reconstructed oocyte; (v)
- transferring the reconstructed zygote or fertilized oocyte into an oviduct of (vi) a recipient female of the same species as the zygote or oocyte; and
  - (vii) allowing the reconstructed zygote or oocyte to develop to term.
- The method of claim 19, wherein the transgene codes for a protein selected from 20. the group consisting of human growth hormone, interferon,  $\beta$ -casein,  $\alpha$ -1 antitrypsin, antithrombin III, collagen, factor VIII, factor IX, factor X, fibrinogen, hyaluronic acid, insulin, lactoferrin, protein C, erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), tissuetype plasminogen activator (tPA), feed additive enzymes, somatotropin and chymotrypsin.

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### Please amend Claim 21 as follows:

21.(Amended). A method of producing a protein, comprising:



- (ii) harvesting hard shell eggs; and
- (iii) isolating the exogenous protein from said eggs.
- 22. The method of claim 21 wherein the exogenous protein is selected from the group consisting of human growth hormone, interferon, β-casein, α-1 antitrypsin, antithrombin III, collagen, factor VIII, factor IX, factor X, fibrinogen,hyaluronic acid, insulin, lactoferrin, protein C, erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), tissue-type plasminogen activator (tPA), feed additive enzymes, somatotropin and chymotrypsin.

Please cancel Claim 23.

- 24. A method of claim 19 wherein the avian is a knock-out or knock-in avian.
- 25. An intact hard shell egg containing less than the normal complement of endogenous proteins found in the egg.
- 26. A reconstituted avian embry prepared by transferring the nucleus of a donor cell into a suitable recipient cell.
- 27. An embryo of claim 26 in which the donor cell is quiescent.



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Please add new Claims 28-33.

28. (New) A method of producing a cloned avian comprising:

- (i) producing a reconstructed zygote by the process of claim 1;
- (ii) transferring the reconstructed zygote into an oviduct of a recipient female of the same species as the zygote; and
- (iii) allowing the econstructed zygote to develop to term.
- 29. (New) The method of claim 28, wherein the cloned avian is selected from the group consisting of chicker back, turkey, quail, ostrich and pheasant.
- 30. (New) A method of producing a cloned avian comprising:
  - (i) producing a reconstructed oocyte by the process of claim 1;
  - (ii) fertilizing the reconstructed oocyte to produce a reconstructed zygote;
  - (ii) transferring the reconstructed zygote into an oviduct of a recipient female of the same species as the zygote; and
  - (iii) allowing the reconstructed zygote to develop to term.
- 31. (New) The method colaim 30, wherein the cloned avian is selected from the group consisting of chicken, duck turkey, quail, ostrich and pheasant.
- 32. (New) A method of preparing a recipient cell comprising the steps of:
  - (i) providing a cell having a nucleus therein;
  - (ii) visualizing the nucleus using light in the near-infrared region; and
  - (iii) ablating the nucleus to provide an enucleated recipient cell.
- 33. (New) The method of claim 32 wherein the nucleus is visualized and ablated via two photon laser scanning microscopy.



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